

## ISOLATION OF JAMESTOWN CANYON AND SNOWSHOE HARE VIRUSES (CALIFORNIA SEROGROUP) FROM *Aedes* MOSQUITOES IN WESTERN MASSACHUSETTS

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**ABSTRACT.** Three isolates of Jamestown Canyon virus and one isolate of snowshoe hare virus (California serogroup) were obtained from adult *Aedes* females collected in western Massachusetts in 1982. Jamestown Canyon virus was isolated from *Aedes abstrusus/punctator* once, and from *Aedes intrudens* twice. Snowshoe hare virus was isolated from *Aedes stimulans* group mosquitoes. La Crosse encephalitis (LAC) virus was not isolated from 1,552 adult *Aedes triseriatus*, nor from 22,557 *Aedes triseriatus* larvae. However, sera from 1/178 eastern chipmunks, 5/31 gray squirrels, and 8/144 white-tailed deer had neutralizing antibody to LAC virus. No sentinel rabbits placed at sites yielding virus isolates seroconverted to CAL viruses in either year.

### INTRODUCTION

California serogroup (CAL) viruses have been intensively studied because of increased recognition of their role as etiologic agents of human disease (Grimstad 1988). Although La Crosse encephalitis (LAC) virus has been the major CAL virus of concern in the eastern U.S., Jamestown Canyon (JC) virus has also been associated with human illness in Michigan (Grimstad et al. 1982), New York and Ontario (Deibel et al. 1983, Srihongse et al. 1984). There has been no systematic survey for CAL viruses in western Massachusetts, although snowshoe hare (SSH), Keystone and several untyped CAL viruses were isolated from mosquitoes during surveillance for eastern equine encephalitis virus in southeast Massachusetts (H. K. Maxfield, Massachusetts Department of Public Health, Encephalitis Field Station, Middleboro; personal communication).

The purpose of this study was to survey western Massachusetts for CAL viruses. Given the proximity of this region to the LAC and JC virus endemic areas in eastern New York State (Grayson et al. 1983, Boromisa and Grayson 1990), we made a special effort to search for these viruses in mosquito, sciurid rodent, and white-tailed deer populations.

### MATERIALS AND METHODS

**Mosquito collection, virus isolation and virus identification.** Adult female mosquitoes were collected at human bait or with dry-ice baited CDC light traps at 17 sites in Franklin, Hampshire,

Hampden and Berkshire counties in western Massachusetts during 1982. Mosquitoes were killed by freezing, sorted by species into pools of 100 or fewer individuals on a chill table and stored at  $-85^{\circ}\text{C}$ . *Aedes triseriatus* (Say) larvae were collected from tree holes and tires at the sites, reared to the fourth instar, pooled into lots of 100 and also stored at  $-85^{\circ}\text{C}$ .

Pools were triturated in 7.5% bovine plasma albumin (BSA) in sterile phosphate-buffered saline (PBS, pH 7.6) supplemented with antibiotics, then centrifuged. Suckling mice (2–4 days old) were inoculated intracerebrally (i.c.) with 0.025 ml of supernatant and observed daily thereafter for signs of illness. Brains of sick mice were harvested, triturated in 0.75% BSA to make 10% (w/v) seeds, centrifuged and passed i.c. to suckling mice. Crude antigens (20% w/v) were prepared by trituration of harvested mouse brains in borate-buffered saline and incubation at  $4^{\circ}\text{C}$  overnight, then centrifuged at 10,000 for 1 h at  $4^{\circ}\text{C}$ . After initial isolation, viruses were re-isolated from the original pools for purposes of confirmation.

Virus isolates were identified first by screening with an indirect immunofluorescent antibody (IFA) procedure described by Boromisa and Grayson (1990). Identifications were confirmed by serum dilution plaque reduction neutralization (SDPRN) tests done on Vero cell monolayers, described by Lindsey et al. (1976) as modified by Boromisa and Grayson (1990). Virus stocks were inoculated onto Vero cell culture monolayers. Virus strains used were a New York strain of LAC virus (74-32813) or prototype strains of LAC, JC, SSH, trivittatus (TVT) and Keystone (KEY) viruses (supplied by the Centers for Disease Control, Fort Collins, CO). In the IFA tests, virus identification was determined by a 2-fold or greater difference in the intensity of immunofluorescence (1–4+) from that of heterologous antibodies. Reference immune sera used in IFA and SDPRN tests were California serogroup virus/type-specific im-

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mune mouse ascitic fluids (IMAFs), and a monoclonal antibody specific for SSH virus (no. 2B3A-2), supplied by the CDC.

**Serosurvey of wild mammals and sentinel rabbits:** Eastern chipmunks (*Tamias striatus*) and gray squirrels (*Sciurus carolinensis*) were live-trapped, anesthetized and bled from the orbital capillary nexus or heart at 9 sites in Franklin, Hampshire, and Berkshire counties in 1980-81. Animals were released after bleeding. Blood of white-tailed deer (*Odocoileus virginianus*) was obtained by aspirating the pool of blood in the abdominal cavity of shot deer brought to checking stations in Berkshire County during the 1980 shotgun season (December 1-6). Sentinel rabbits were set out in secured metal cages at 8 of the 9 trapping sites. In 1981, 14 rabbits were placed in early July and removed in late September; in 1982, 10 rabbits were placed in early May and removed in early July. The rabbits were bled (ear vein or heart) before placement and weekly thereafter.

Mammal blood was centrifuged, and the sera separated and stored at -40°C. Sera were screened for antibody to CAL viruses in neutralization tests done in suckling mice using a New York strain of LAC virus as antigen. Rabbit sera were also screened in neutralization tests with this LAC virus isolate and a New York State isolate of JC virus (78-30641) as antigen. Sera that were positive in screenings were titrated in neutralization index tests in suckling mice, using a constant-serum, varying-virus dilution method (Lennette and Schmidt 1969). Sera neutralizing at least 1.7 logs of virus were considered positive.

## RESULTS

**Virus isolations from mosquitoes:** The results of mosquito collections are shown in Table 1. In 1981, 1,732 mosquitoes were collected in 92 pools. In 1982, 42,515 mosquitoes were collected in 669 pools. Collections consisted mainly of spring *Aedes* and *Aedes triseriatus* larvae and adults. Many specimens were rubbed, so that some similar species were lumped into species groups (see Table 1).

Four CAL viruses were isolated from mosquitoes (Table 2). Snowshoe hare was isolated once from *Aedes stimulans* (Walker) group, which probably also included *Aedes excrucians* (Walker) and *Aedes fitchii* (Felt and Young). These mosquitoes were collected on June 9, 1982, in the Lawrence Swamp Conservation Area, Amherst, Hampshire County. Jamestown Canyon virus was isolated 3 times: from *Aedes intrudens* Dyar collected on June 10, 1982, in Lawrence Swamp; from *Aedes abstratus* (Felt

Table 1. Mosquito collections from Franklin, Hampshire, Hampden and Berkshire counties, western Massachusetts, 1981-82

Species	No. mosquitoes	No. pools
<i>Ae. abstratus/punctor</i>	3,844	68
<i>Ae. aurifer</i>	95	13
<i>Ae. canadensis</i>	1,222	54
<i>Ae. cinereus</i>	151	19
<i>Ae. communis</i>	6,001	87
<i>Ae. diantus/decticus</i>	370	26
<i>Ae. implicatus</i>	3	1
<i>Ae. intrudens</i>	3,060	57
<i>Ae. provocans</i>	2,497	45
<i>Ae. stimulans</i> group	2,264	53
<i>Ae. triseriatus</i> adults	1,552	40
<i>Ae. triseriatus</i> larvae	22,557	243
<i>Ae. trivittatus</i>	88	12
<i>Ae. vexans</i>	461	23
<i>An. quadrimaculatus</i>	2	2
<i>An. punctipennis</i>	12	7
<i>An. walkeri</i>	1	1
<i>Cq. perturbans</i>	17	6
<i>Cx. pipiens/restuans</i>	47	2
<i>Cs. morsitans</i>	3	2
Total	44,247	761

and Young) group (including *Aedes punctor* (Kirby) collected on June 22, 1982, in Lawrence Swamp); and from *Ae. intrudens* collected on June 29, 1982, in Warwick State Forest, Franklin County. All isolates were reisolated and identified from the original pools.

A summary of virus isolation and identification from these mosquito pools is given in Table 2. In IFA screen tests, three isolates (nos. 247, 471 and 555) gave strongly positive (3-4+) reactions with JC IMAF; negative or weak reactions ( $\leq 1+$ ) were obtained with the other reference IMAFs. Isolate no. 235 exhibited positive reactions (2-3+) with LAC and SSH IMAF (which were strongly cross-reactive) but did not react with IMAFs to JC, KEY or TVT viruses. In the SDPRN tests (Table 2), the number of plaques formed in control wells ranged from 82 to 139. The titer of the JC IMAF for 3 isolates (nos. 247, 471 and 555) was at least 4-fold higher than the other CAL serogroup IMAFs, confirming their identity as JC viruses. For isolate no. 235, there was cross-reaction between LAC and SSH IMAF at a titer of 1:160. However, the SSH monoclonal antibody had a titer of 1:2,560 for this isolate in the SDPRN test, while this antibody had  $<1:20$  titers with the heterologous CAL viruses and with New York LAC strain no. 74-32813. We conclude that isolate no. 235 was SSH virus.

**Serosurvey of wild mammals and sentinel rabbits:** No sentinel rabbits seroconverted to LAC or JC viruses in either 1981 or 1982. One of 178

Table 2. Isolations of Jamestown Canyon and snowshoe hare viruses from *Aedes* mosquitoes collected in Western Massachusetts, 1982. Results of serum dilution plaque reduction neutralization tests, given as reciprocal titers in reactions with virus type-specific hyperimmune mouse ascitic fluids

Mosquito species	Isolate no.	Pool size	MFIR	Reciprocal serum dilution plaque reduction neutralization titers						
				JC	TVT	LAC	SSH	SSH Mab	KEY	N
<i>Ae. stimulans</i> group	235	100	1:2,264	<20	20	160	160	2,560	40	<20
<i>Ae. intrudens</i>	247	55	1:1,530	80	20	<20	<20	NT	<20	<20
<i>Ae. abserratus/punctor</i>	471	17	1:3,844	80	20	<20	<20	NT	<20	<20
<i>Ae. intrudens</i>	555	100	1:1,530	80	20	20	<20	NT	<20	<20

MFIR, minimum field infection rate; JC, Jamestown Canyon; TVT, trivittatus; LAC, La Crosse; SSH, snowshoe hare; KEY, Keystone; N, no viral antigen used (i.e., negative control); Mab, monoclonal antibody; NT, not tested.

(0.6%) eastern chipmunks, 5/31 (16%) gray squirrels and 8/144 (6%) white-tailed deer had neutralizing antibody to LAC virus. Positive animals came from widely separated sites.

## DISCUSSION

Jamestown Canyon virus has been isolated from a variety of biting flies, including Tabanidae and Culicidae (Turell and LeDuc 1983, Grimstad 1988). In the eastern U.S., JC virus has been associated with the *Aedes communis* (De Geer) group (i.e., "dark-legged" spring *Aedes*), *Ae. abserratus* and *Ae. stimulans*, as well as *Anopheles* spp. and *Ae. triseriatus* (Grimstad 1988). Recent studies in northern Michigan (Heard et al. 1990) and New York State (Boromisa and Grayson 1990) implicate *Aedes provocans* (Walker) as a vector of JC virus; minimum field infection rates for that species were high in both of those studies, ranging from 1:27 to 1:714, depending upon the site and year. We did not isolate JC virus from 2,497 *Ae. provocans*, even though collections were made at sites where JC virus was isolated from *Ae. intrudens* and *Ae. abserratus/punctor*. Our data indicate that *Ae. intrudens* and *Ae. abserratus/punctor* may be enzootic vectors of JC virus in western Massachusetts. Heard et al. (1990) isolated JC virus from *Ae. intrudens* in northern Michigan, while Main et al. (1979) isolated JC virus from *Ae. abserratus* in Connecticut. However, the minimum field infection rates we obtained were considerably lower than in those studies.

Isolation of SSH virus from *Ae. stimulans* group mosquitoes in our study is consistent with surveys in New York, where this virus was isolated numerous times from *Ae. stimulans* group and *Ae. canadensis* (Grayson et al. 1983). Previously, SSH virus was isolated from *Ae. canadensis* in Bristol County in southeastern Massachusetts, in 1968 (H. K. Maxfield, Massachusetts Department of Public Health, Encephalitis

Field Station, Middleboro; personal communication).

Results of the serosurvey of eastern chipmunks, gray squirrels and white-tailed deer showed low rates of exposure of these mammals to CAL viruses. Use of LAC virus as antigen in our neutralization tests on sera from these animals would detect antibody specifically to LAC virus or SSH virus, or possibly to heterotypic CAL viruses. Thus, we cannot conclude definitively that the seropositive mammals had been exposed to LAC virus. Considering that LAC virus was not isolated from mosquitoes collected in area where seropositive chipmunks and squirrels were trapped, we think it more likely that they were exposed to another CAL virus, probably SSH.

Western Massachusetts seems to be a likely area for CAL viruses, including LAC virus, to occur. Forest regrowth and maturation (MacConnell 1975) created extensive woodland habitat for spring *Aedes* and *Aedes triseriatus*, as well as for sciurid rodents and white-tailed deer. Also, western Massachusetts abuts the CAL virus endemic areas of eastern New York State.

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## REFERENCES CITED

- Boromisa, R. D. and M. A. Grayson. 1990. Incrimination of *Aedes provocans* as a vector of Jamestown

- Canyon virus in an enzootic focus of northeastern New York. *J. Am. Mosq. Control Assoc.* 6:504-509.
- Deibel, R., S. Srihongse, M. A. Grayson, P. R. Grimstad, J. S. Mahdy, H. Artsob and C. H. Calisher. 1983. Jamestown Canyon virus: the etiologic agent of an emerging human disease, pp. 313-325. *In*: C. H. Calisher and W. H. Thompson (eds.), *California serogroup viruses*. A. R. Liss, New York.
- Grayson, M. A., S. Srihongse, R. Deibel and C. H. Calisher. 1983. California serogroup viruses in New York State: a retrospective analysis of subtype patterns and their epidemiologic significance, 1965-1981, pp. 257-267. *In*: C. H. Calisher and W. H. Thompson (eds.), *California serogroup viruses*. A. R. Liss, New York.
- Grimstad, P. R. 1988. California group virus disease, pp. 99-136. *In*: T. P. Monath (ed.), *The arboviruses: epidemiology and ecology*, vol. 2. CRC Press, Boca Raton, FL.
- Grimstad, P. R., C. L. Shabino, C. H. Calisher and R. J. Waldman. 1982. A case of encephalitis in a human associated with a serological rise to Jamestown Canyon virus. *Am. J. Trop. Med. Hyg.* 31:1238-1244.
- Heard, P. B., M. Zhang and P. R. Grimstad. 1990. Isolation of Jamestown Canyon virus (California serogroup) from *Aedes* mosquitoes in an enzootic focus in Michigan. *J. Am. Mosq. Control Assoc.* 6:461-468.
- Lenette, E. H. and N. J. Schmidt. 1969. Diagnostic procedures for viral and rickettsial infections. *Am. Public Health Assoc.*, New York.
- Lindsey, H. S., C. H. Calisher and J. H. Mathews. 1976. Serum dilution neutralization test for California group virus identification and serology. *J. Clin. Microbiol.* 4:503-510.
- MacConnell, W. P. 1975. Remote sensing 20 years of change in Hampshire County, Massachusetts, 1952-1972. *Mass. Agric. Exp. Stn. Res. Bull.* 628.
- Main, A. J., S. E. Brown, R. D. Wallis and J. Elston. 1979. Arbovirus surveillance in Connecticut. II. California serogroup. *Mosq. News* 39:552-559.
- Srihongse, S., M. A. Grayson and R. Deibel. 1984. California serogroup viruses in New York State: the role of subtypes in human infections. *Am. J. Trop. Med. Hyg.* 33:1218-1227.
- Turell, M. J. and J. W. LeDuc. 1983. The role of mosquitoes in the natural history of California serogroup viruses, pp. 43-55. *In*: C. H. Calisher and W. H. Thompson (eds.), *California serogroup viruses*. A. R. Liss, New York.